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Impact of broccoli antioxidant activity on liver fibrosis induced by carbon tetrachloride in rats

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ABSTRACT

The excessive extracellular matrix proteins (ECM) caused liver fibrosis. The research was designed to evaluate the impact of antioxidants in broccoli to improve the liver of rats injured by carbon tetrachloride (CCl4). The study included 32 healthy albino rats (Reference Na 83-18), weighing about (170-200gm) were divided into four experimental groups: Group1 (n=7): as normal control, the rats fed a basal diet. Groups from 2 to 4 (n=23): 1ml/kg of CCl_4 used to inject the rats intraperitoneally mixed by corn oil at 1:1 twice weekly for three weeks to induced fibrosis. Group 2 (n=8): rats administrated with CCl4 at dose 1ml/kg as a CCl4-treated group. Group 3 (n=7) Group 4 (n=8): used the broccoli extract to feed rats for 15days at dose 100mg/kg.b.w and 200mg/kg.b.w. The results reported that group 2 significantly increases in the liver enzymes (ALT, AST) and in the Malondialdehyde (MDA), a decrease in body weight (B.W) Glutathione-S-Transferase (GST) and vitamin D levels compared to the normal group. Broccoli antioxidants improved and increased B.W and decrease the ALT, AST, and MDA. Also, the GST and VitD levels were increase compared to group 2. Histopathological investigation of group 2, it showed damage and necrosis in hepatocytes, after the treatment, this damage became less and tend to normal liver cell. The study concluded that the protective effects of broccoli antioxidants may improve against liver fibrosis. Therefore, we recommended consuming the broccoli within daily meals to enhance antioxidant capacity.

Keywords: Liver fibrosis, Broccoli, Vitamin D, CCl_4, Rats.

1. INTRODUCTION

A common pathological disorder following the long-standing liver injury that develops from chronic wound healing response is liver fibrosis (Böttcher & Pinzani, 2017). It is a dynamic cellular process characterized by activation of the hepatic myofibroblasts (MFs) and excess accumulation of extracellular matrix (ECM) components (Novo *et al.*, 2014). Non-alcoholic fatty liver disease (NAFLD), Viral hepatitis [HBV and HCV] and autoimmune hepatitis are various factors that caused liver fibrosis (Sun & Kisseleva, 2015). NAFLD

can progress to liver fibrosis (Singh *et al.*, 2015), its prevalence was estimated at 8,451,000 (25.7%) in Saudi Arabia, by 2030 it was projected to increase by 48% (Alswat *et al.*, 2018). An unbalanced rise in reactive oxygen species (ROS) with overall pro-oxidant activity occurred in many hepatic disease processes that affect their structure and function (Poli, 2000).

In recent years, a large number of natural plants have attempted to eliminate the action of ethanol or CCl4-induced hepatic damage in animal models, flavonoids and polyphenols are the bioactive compounds that are responsible for relieving oxidative stress (Li *et al.*, 2015). Broccoli found to have a high antioxidant activity correlated positively with total phenolics (Moreno *et al.*, 2006). The key bioactive component which suggested to be responsible for most of the activities of broccoli is Isothiocyanates called sulforaphane (SF) (Khalaj *et al.*, 2013).

The objective of this study is to determine the effect of antioxidants from the aqueous ethanol extract of broccoli on rats that have liver fibrosis caused by CCl4 and explain the action of bioactive compounds in broccoli in defense the oxygen free radical during the disease process.

2. MATERIALS AND METHODS

Plant Materials

From the local market, we purchased the fresh broccoli.

Methods

Preparation the Ethanolic Broccoli Extract (BE)

According to (Subramanian, 2011) the broccoli was obtained from the local market at Jeddah. Fresh flowering portion of broccoli was collected, washed and dried under shade for one week and pulverized in a mechanical grinder (Subramanian, 2011). About 800g of dried powder broccoli was extracted by ethanol solvent mixed with water at ratio 9:1 (v/v ethanol/water) three times every 48h, then, the extract was filtered and collected by rotary evaporator system machine to evaporate the solvent.

Broccoli Extract (BE) dose preparation

The amount of dried ethanolic BE was calculated and suspended in 0.5% w/v of sodium- carboxymethylcellulose (CMC) in normal saline solution to prepare the doses (100, 200mg/kg/ml.).

Animals

This research was accepted by Unit of Biomedical Ethics Research Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia (Reference No. 83-19). In March 2019, the animal unit of KFMRC, King Abdulaziz University, has obtained healthy male waster albino rats (n=32 rats) weighing around (170-200gm). The rats were divided into four experimental groups, housed in clean and sterile polyvinyl cages (8 rats/cage), preserved on standard laboratory water ad libitum and pellet diet; they protected in a temperature at 22–24 °C, relative humidity (50-55%) and 12 h dark/light cycle.

Experimental Design

Group 1: normal control Group (n=7*): rats were fed only water ad libitum pellet diet and.
Group 2: CCl4-treated group(n=8): rats have injected interperitoneally (IP) with CCl4 at the dose of 1ml/kg body weight (1:1 v/v CCl4 /Corn oil) twice weekly (Sunday, Wednesday) for three weeks to induced hepatotoxicity. Kept as the injury group.
Group 3: CCl4 +BE 100mg/kg (n=7*): rats treated orally with BE 100mg/kg for 15 days
Group 4: CCl4 +BE 200mg/kg (n=8): rats fed with BE 200mg/kg orally for 15days.
1Dose of broccoli extract according to El-Baz (2012)
*Two rats died in the first week of experiment one from group1 and another from group 3.

Blood Collection and Histopathological Examination

After five weeks, 5ml of blood was withdrawn from the retro-orbital plexus of the rat's eyes under euthanized following anesthesia by diethyl ether. Liver tissue specimens were rinsed in normal saline solution and weighted then placed in formalin buffer 10%. The samples were undergone in the pathology lab to routine histological, King Abdulaziz University Hospital (KAUH), Jeddah.

Measurement of Biochemical Parameters

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were detected by a colorimetric assay kit (QUIMICA CLINICA APLICADA S.A, SPAIN) IFCC method according (Bergmeyer, 1980). Glutathione-S-transferase (GST) level was assayed using

colorimetric assay kit (Habig, Pabst & Jakoby, 1974), (Elabscience, USA) and the malonaldehyde ELISA kit was carried out according to (Elabscience, USA). The total vitamin D serum level was determined by the ELISA kit (Siemens Healthcare Diagnostics Inc. USA).

Statistical Analysis

The statistical data analysis was carried out using the SPSS (Statistical Program for Social Sciences).

3. RESULTS

The importance of antioxidant plants is the hepatocellular defense against free radicals, which has caused liver damage. Broccolis include many bioactive compounds that may enhance the hepatic injury induced by CCl4 administration which used in laboratory experiment. The results were shown the body weight (BW) of rats significantly decreased (P<0.05) in group 2 (mean =251.38gm) compared with the control group (mean=336.86gm). However, it was increased in group 3 (mean=341.14gm) and in group 4 (308.50gm) compared to CCl_4 group (Table 1).

Table 1 Comparison the rat's liver weight mean; body weight means and liver index in each experimental group.

Experimental Group	Liver weight mean (g)	Body Weight mean (g)	Liver Index % (Liver weight/ Body weight*100)
Group 1 (Control)	11.81	336.86	3.5
Group 2 (CCl_4 treated)	10.79	251.38	4.29
Group 3	11.66	341.14	3.41
Group 4	10.41	308.50	3.37

The levels of ALT and AST were significant reduction in group 3 and group 4 compared to group 2 and their activities elevated in CCl4 group compared to control group (P-value < 0.01) (Figure 1). Lipid peroxidation (MDA) was increased in group 2 compared to control, which indicated the presence of oxidative stress in rat liver. However, after we used broccoli extract treatment, we showed that it enhanced the MDA concertation activity. However, the serum level of GST decreased in group 2 compared to control also, GST level in groups 3 and 4, tend to be normalized after broccoli treatment. Hepatotoxicity in rat's liver by CCl4 caused the decreased level of Vit D in group 2 compared to the control. However, the natural antioxidant of broccoli extract may improve the Vit D level; we showed that in group 3 and group 4 compared to the CCl4 group (Table 2 & 3).

Table 2 Serum of liver enzymes activity, antioxidants enzymes, malondialdehyde level and vitamin D level in Group 3 rats

Group 3	ALT (U/L)	AST (U/L)	MDA (ng/ml)	GST (U/ml)	SOD (U/ml0	Vit D (nmol/L)
Before Experiment	15.20±14.24	58.92±44.44	63.18±20.96	35.49±11.95	42.30±3.44	188.70±2.26
AfterCCl_4 dose period	48.31±51.00	80.27±24.31	77.22±7.72	21.69±20.32	42.97±1.87	165.80±6.22
End the Experiment	22.94±5.07	41.30±15.16	64.74±43.02	27.61±0.80	44.34±1.07	212.15±15.20

Table 3 Serum of liver enzymes activity, antioxidants enzymes, malondialdehyde level and vitamin D level in Group 4 rats

Group 4	ALT (U/L)	AST (U/L)	MDA (ng/ml)	GST (U/ml)	SOD (U/ml0	Vit D (nmol/L)
Before Experiment	16.83±4.20	39.89±23.62	51.78±18.07	17.19±9.96	40.54±0.06	106.30±26.02
After CCl_4 dose period	116.31±107.40	141.80±84.32	46.02±1.10	47.33±1.59	38.90±1.63	105.60±17.68
End the Experiment	16.69±6.35	39.64±8.41	60.22±4.04	27.61±0.00	41.64±0.62	119.05±28.78

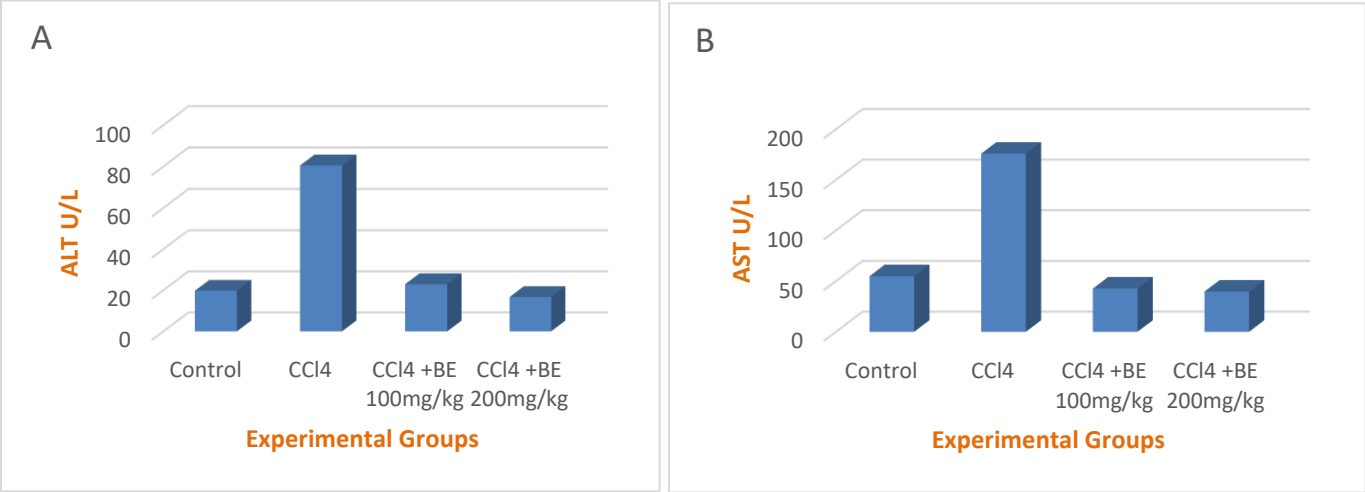

Figure 1 Effect of BE on CCl4-induced elevations in serum liver enzyme activities in rats. A) ALT level in rats treated with CCl_4 and after used the BE for 15 days with different doses. B) AST level in rats treated with CCl_4 and after used the BE for 15 days with different doses. BE, broccoli extract; CCl_4, carbon tetrachloride; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Histopathological Examination

In experimental models CCl4-induced hepatotoxicity was extensively used to understand the cellular mechanisms behind hepatic damage and to determine the therapeutic potential of broccoli antioxidants (figure 2).

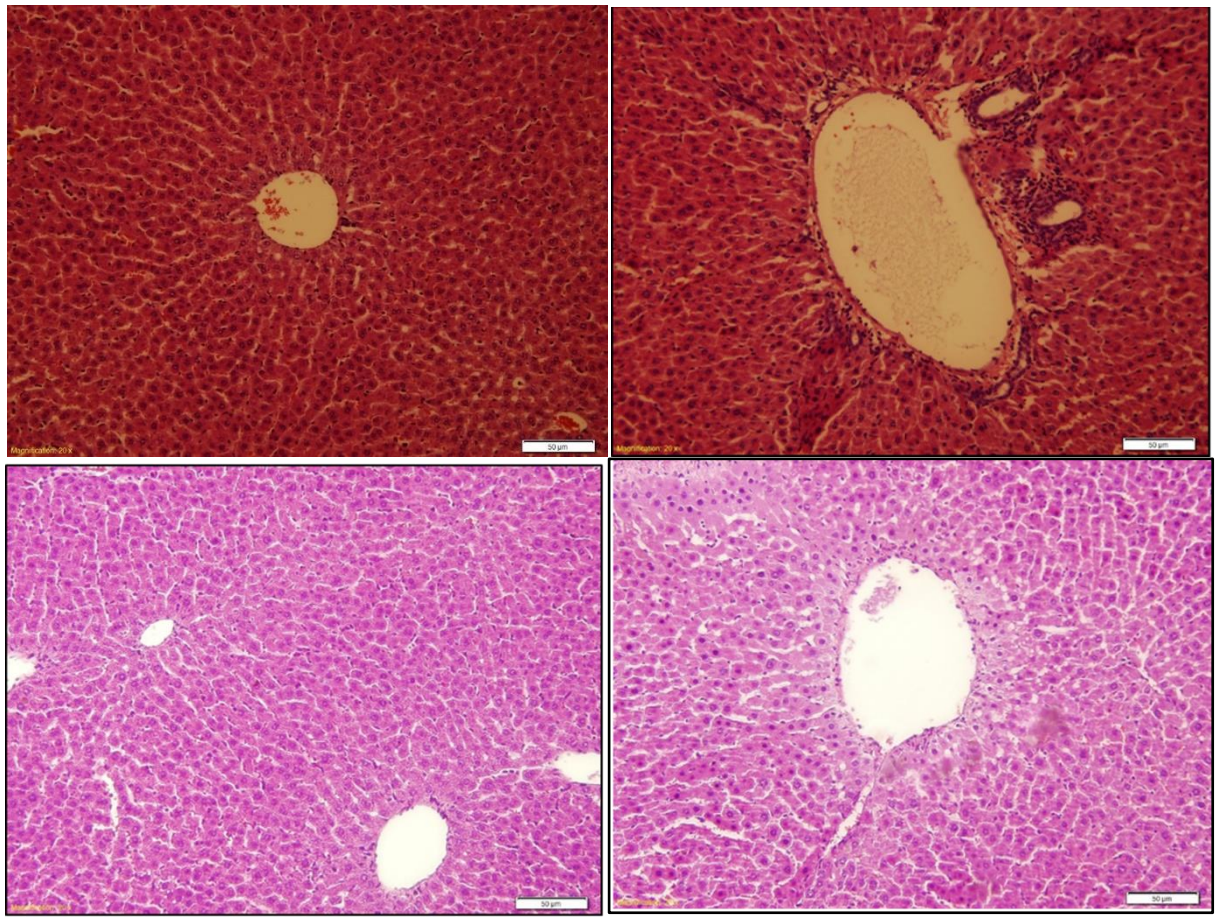

Figure 2 Effects of BE on CCl4-induced histopathological alterations. A) Rat liver of normal control rat (H & E, 20x·), B) Rat liver of CCl4 -treated rat (H & E, 20x), C) Rat liver of CCl4 + broccoli extract 100mg/kg (H & E, 20x)) and D) Rat liver of broccoli extract 200mg/kg and CCl4 -treated rat (H & E, 20x).

4. DISCUSSION

The process that occurs after exposure to drugs and toxicity than lead to hepatic injuries is liver fibrosis (Duval *et al.*, 2015). As a standout of hepatotoxins, CCl4 is commonly used to induce experimental models (Zarezade *et al.*, 2018). Antioxidant administration prevents lipid peroxidation, oxidative stress, and liver fibrosis (Parola & Robino, 2001). This research examined the effect of broccoli on fibrosis in rats treated with CCl4 to induce experimental fibrosis. Body weight was shown to decrease significantly by (25.38%) in group 2 compared to group 1. These results were accepted by Kikuchi et al. (2015), who mentioned the loss of body weight in the CCl4 group. Ethanolic extract of broccoli increased the rat body weight with liver fibrosis (Kikuchi *et al.*, 2015).

In group 3, rats' body weight increased significantly by 35.70% while in group 4 rats is increased by 22.72% compared with group 2. The common indicators are used as the markers, increased levels of cytoplasmic enzymes (ALT and AST) of liver damage because these are released from cytoplasm liver cells into the blood serum (Wang *et al.*, 2018). In ALT and AST, we observed a significant increase in serum of CCl4 group (80.51U/L, 175.81U/L respectively) compared to normal control. The findings obtained were accepted with Ashoush et al. (2013), which showed a significant increase in the ALT and AST serum group treated with CCl4 compared to the control group (Ashoush, El-Batawy & El-Shourbagy, 2013).

CCl4 administration into rats resulted in hepatocellular damage; it produced an increase of 18.9% in the MDA level in the CCl4 group compared to control. Also, it caused decreases in GST activity by 22.03% and total VitD level by 5.5% compared to the control. The administration of broccoli extract has a protective effect that has been shown to significantly reduce the elevated levels of ALT and AST, reducing serum MDA by 1.87% and 13.5%, respectively, in group 3 and group 4, relative to the CCl4 group. BE increased the level of GST in group 3 by 24.5% and in group 4 by 31.4% in serum relative to the CCl4 group. However, the rats in group 3 that treated with BE increased the serum VitD concentration by 22.35% and by 5.58% in group 4 relative to the CCl4 group.

Histopathological Evaluation

Figure (2, A) in the normal group, showed the natural histological structure of the portal area and central veins, surrounding hepatocytes. Also, in figure (2, B) we showed severe vascular degenerative changes as well as mononuclear inflammatory cells and hepatic parenchyma infiltrations and appeared severe congestion of blood vessels in the portal area. The pathological alteration and slight congestion of the hepatic vein decreased in that figure (2, C and D) with fewer cells suffered from vacuolar degenerative changes. These findings were accepted with Al-Howiriny (2008), who reported that broccoli can protect against histopathological in the liver of CCl4-induced hepatic injury in rats (Al-Howiriny, 2008).

5. CONCLUSION AND RECOMMENDATIONS

Broccoli flower supplementation has benefits on the liver and can improve antioxidant enzymes in the defense of disease-based oxidative stress. A further study to demonstrate the mechanism of these cruciferous vegetables should be carried out.

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Author Contributions

Rawan A Alsahafi: I'm Master student who did the experiment and wrote the paper. Prof. Dr. Jalaluddin Awlia: My supervisor who review and guide me to do this project. Dr. Maryam Al-Ghamdi: My co-supervisor also assists me and supports this project to complete.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical approval for animal studies

This study was approved (ethical approval number. 83-19) by the Unit of Biomedical Ethics Research Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Data and materials availability

All data associated with this study are present in the paper.

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